Red Wine Phenolic Compounds Reduce Plasma Lipids and Apolipoprotein B and Prevent Early Aortic Atherosclerosis in Hypercholesterolemic Golden Syrian Hamsters (*Mesocricetus auratus*)

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ABSTRACT The effects of a red wine phenolic extract (PE) on plasma lipoproteins and early atherosclerosis were studied in hamsters. Hamsters (*n* = 32) were divided into 4 groups of 8 and fed an atherogenic diet for 8 wk. They received by force-feeding 7.14 mL/(kg·d) PE in 2.6 mol/L ethanol (E + PE) or PE in water (W + PE), mimicking a moderate consumption of red wine or alcohol-free red wine (30.4 mg/(kg·d)), or 2.6 mol/L ethanol (E-PE) or water (W-PE) as their respective controls. Plasma cholesterol and triglyceride concentrations were lower in groups that consumed PE. The decrease in plasma apolipoprotein (Apo) B concentration was due mainly to PE and was significantly lower in Group E + PE than in Group E-PE (−7.5%) and in Group W + PE than in Group W-PE (−40%). Apo-A1 was not affected. PE significantly increased plasma antioxidant capacity by 9% in Group E + PE and 18% in Group W + PE compared with their respective controls. Liver glutathione peroxidase activity was 67% greater in the group receiving PE in water compared with the group given water; there was no effect when PE was given in ethanol relative to its control. Aortic fatty streak area (AFSA) was significantly reduced in the groups receiving PE in ethanol (−32%) or PE in water (−29%) in comparison with their respective controls. Ethanol significantly reduced AFSA by 60% (Group E-PE vs. Group W-PE) or 62% (Group E + PE vs. Group W + PE). These data suggest that ethanol is a complementary component of phenolics in the benefits of red wine for hamsters and that chronic ingestion of PE in ethanol prevents the development of atherosclerosis through several mechanisms. With moderate consumption of red wine, ethanol can improve the effects of phenolic compounds. However, alcohol-free red wine appears to be a very good alternative to red wine. J. Nutr. 132: 1207–1213, 2002.

KEY WORDS: atherosclerosis • red wine • phenolic compounds • ethanol • hamsters

Many epidemiology studies have demonstrated that moderate consumption of alcoholic beverages is associated with reduced mortality and risk of cardiovascular disease (CVD) (1–3). Wine contains phenolic compounds, which have been reported to have a number of antioxidant properties (4) that may contribute to a reduced risk of CVD in wine drinkers. The greatest degree of cardioprotection is related to ingestion of red wine rather than white wine, beer or spirits (5–9). Renaud and de Lorgeril (10) demonstrated that the consumption of red wine rather than white wine, beer or spirits (5–9). Renaud and de Lorgeril (10) demonstrated that the consumption of red wine appears to be a very good alternative to red wine.

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cause the antioxidant vitamin E (22) and vitamin E plus catechin (23) have been reported to inhibit the development of aortic lesions in golden Syrian hamsters, we studied the effects of red wine phenolic compounds in this model. Feeding hamsters a cholesterol-supplemented diet associated with antioxidant (vitamin C, vitamin E and selenium) deficiency produces dyslipidemia and arterial lesions that have been shown to be similar in many respects to those found in human atheroma (24, 25). This model was also chosen because of its responsiveness to plasma cholesterol-lowering and antiatherogenic interventions (26–28). Moreover, hamsters have a plasma lipoprotein distribution similar to that of humans. The aim of the present study was to determine whether phenolic compounds of red wine or ethanol administered separately or in combination affected the development of atherosclerosis. To do so, an aortic wall response to a high cholesterol/low antioxidant diet was triggered in Syrian hamsters to induce fatty streak formation and atherosclerosis emergence; we then evaluated the possible preventive effect of the administration of a red wine phenolic extract solubilized in water or ethanol.

MATERIALS AND METHODS

Preparation of red wine phenolic extract (PE). The PE dry powder from red French wine (Corbières A. O. C.) was prepared according to the following procedure: briefly, phenolic compounds were adsorbed on a preparative column, then alcohol desorbed; the alcoholic-eluent was gently evaporated; the concentrated residue was lyophilized and finely sprayed to obtain the PE dry powder. One liter of red wine produced 2.9 g of PE, which contained 471 mg/kg of total phenolic compounds expressed as gallic acid. Phenolic levels in PE were measured by HPLC. The extract contained 8.6 mg/g catechin, 8.7 mg/g epicatechin, dimers (B1: 6.9 mg/g, B2: 8.0 mg/g, B3: 20.7 mg/g and B4: 0.7 mg/g), anthocyanins (malvidin-3-glucoside: 11.7 mg/g, pelargonidin-3-glucoside: 0.66 mg/g, and cyanidin-3-glucoside: 0.06 mg/g) and phenolic acids (gallic acid: 5.0 mg/g, caffeic acid: 2.5 mg/g, and caftaric acid: 12.5 mg/g).

Standards and HPLC analysis. (+)-Catechin and (-)-epicatechin were obtained from Aldrich (St. Quentin Fallavier, France). Procyanidins dimers B1, B2, B3 and B4 were obtained from grape seeds as previously reported (29). HPLC analysis with UV detection was performed with a gradient of acetonitrile; solvent A, 0.1 mol/L orthophosphoric acid adjusted to pH 1.5. Elution was performed with a gradient of 0.7 ml/min. The solvents used for separation (30) were as follows: solvent A, 50 mmol/L dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B, 20% A with 80% acetonitrile; solvent C, 200 mmol/L orthophosphoric acid adjusted with ammonia to pH 1.5. Elution was performed with a gradient previously described (29).

Animals. Male golden Syrian hamsters (n = 32; Janvier, Le Genest-St-Isle, France) weighing 60–80 g were randomly divided into four groups of eight with approximately equal mean group body weights. Hamsters were housed in groups of 8 per plastic cage in a temperature controlled room (23 ± 1°C) subjected to a 12-h light:dark cycle (lights on at 0700 h) with free access to both food and water. Hamsters were handled according to the guidelines of the Committee on Animal Care at the University of Montpellier and NIH guidelines (31).

Diets and feeding procedures. Hamsters were fed a semipurified pellet diet in which the cholesterol concentration had been set at 0.5 g/100 g containing 15 g/100 g lard (Table 1). This atherogenic diet was free of selenium, vitamin C and vitamin E. Hamsters were given food daily for 8 wk and uneaten food was weighed daily. Each group of hamsters was additionally force-fed daily either a solution of PE in 2.6 mol/L ethanol (Group E + PE) or a solution of PE in water (Group W + PE) or 2.6 mol/L ethanol (Group E-PE) or tap water (Group W-PE).

The volume of solutions force-fed was adjusted daily to the weight of hamsters: it was established by extrapolating 500 mL/d average wine consumption, i.e., ~2 glasses per meal (wine containing 2 g/L total phenolic compounds) for a 70-kg human to the equivalent for the daily weight of hamsters. This represents a volume of 7.14 mL/kg d). Because PE contained 470 mg/g phenolic compounds (as gallic acid equivalent), hamsters from Groups E + PE and W + PE received 30.4 mg PE/(kg d) dissolved in the above-mentioned volume of either 2.6 mol/L ethanol or water.

Analytical procedures. At the end of the 8-wk experimental period, hamsters were deprived of food for 18 h and were anesthetized with an intraperitoneal injection of pentobarbital (60 g/L at a dosage of 60 mg/kg body). Blood was drawn by cardiac puncture with heparin-moistened syringes and plasma was prepared by centrifugation at 2000 g for 10 min at 4°C, then stored at −80°C until analysis. Plasma total cholesterol (TC), HDL cholesterol (HDL-C) and triglycerides (TG) were determined by commercially available enzymatic methods (respectively nos. 401, 352–4, and 343, Sigma Chemicals, Saint Quentin Fallavier, France). Plasma VLDL and LDL cholesterol (nonHDL-C) were precipitated with phosphotungstate reagent (32) and HDL-C was measured in the supernatant. Plasma apolipoprotein (Apo) A1 and Apo-B concentrations were determined using Sigma turbidimetric immunoassay kits (nos. 356 and 357, respectively) as previously described (33, 34). The liver was perfused with 0.15 mol/L KCl to remove residual blood, rapidly excised, rinsed in ice cold saline, blotted dry, weighed, sectioned for analysis and stored in liquid nitrogen. Liver was homogenized in 5 volumes ice-cold 0.1 mol/L potassium phosphate buffer (pH 7.4) and the homogenate was centrifuged at 13000 g for 15 min at 4°C. The supernatant was then centrifuged at 105000 g for 60 min at 4°C and cytosols were stored at −80°C for subsequent assay of Se-dependent glutathione peroxidase activity (Se-GSHPx). This activity was measured according to Wendel (35) using 0.2 mmol/L hydrogen peroxide as the substrate and including 1.0 mmol/L sodium azide to inhibit catalase, so that only Se-GSHPx activity was measured. The cytosolic protein content was determined by a commercial protein assay (Sigma) according to Smith et al. (36), using bovine serum albumin as a standard.

| TABLE 1
Composition of the diet
<table>
<thead>
<tr>
<th>Diet ingredient</th>
<th>Experimental diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
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<tr>
<td>α-Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>39</td>
</tr>
<tr>
<td>Sucrose</td>
<td>154</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix 1</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix 2</td>
<td>10</td>
</tr>
<tr>
<td>Lard</td>
<td>150</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5</td>
</tr>
</tbody>
</table>

1 Mineral mixture contained (mg/kg diet): CaHPO 4, 17.20; KCl, 4000; NaCl, 4000; MgO, 420; MgSO 4, 2.000; Fe 2O 3, 1200; FeSO 4 · 7H 2O, 200; trace elements, 400 (MnSO 4 · H 2O, 98; CuSO 4 · 5H 2O, 20; ZnSO 4 · 7H 2O, 80; CoSO 4 · 7H 2O, 0.16; KI, 0.32; sufficient starch to bring to 40 g (per kg diet).

2 Vitamin mixture containing (mg/kg diet): retinol, 12; cholecalciferol, 0.125; thiamin, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyancobalamin, 0.1; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; -aminobenzoic acid, 100; biotin, 0.6; sufficient starch to bring to 20 g (per kg diet).
Effects of force-feeding red wine phenolic extract (PE) in 2.6 mol/L ethanol (E + PE), red wine phenolic extract in water (W + PE), 2.6 mol/L ethanol (E-PE) or water (W-PE) on body weight and food intake of hamsters fed an atherogenic diet

Aortic tissue processing. After blood collection and liver removal, the intact aorta was first perfused with PBS containing 1 mmol/L CaCl₂ and 830 mmol/L paraformaldehyde and 150 mmol/L glutaraldehyde for the fixation of the vasculature as described by Simionescu et al. (38). The aorta was carefully dissected between sigmoid valves and stored at 4°C until staining. The aortic arches were then rinsed in 11.7 mol/L isopropyl alcohol and placed in distilled water. Each aortic arch was then directly displayed en face on a glass slide, endothelium side up, covered with Aquamount mounting medium and cover slips and observed en face by light microscopy. All segments were photographed using a video digitizer. The Oil red O stained area was analyzed quantitatively using a computer-assisted morphometry system and expressed as a percentage of the total area surveyed.

Statistical analyses. Data are shown as the means ± SEM, n = 8 measurements/group. Data were subjected to logarithmic transformation where necessary to achieve homogeneity of variances. Statistical analysis of the data were carried out using the Stat View IV software (Abacus Concepts, Berkeley, CA) by two-way ANOVA with liquid matrix (water, alcohol) and PE (with, without) as main effects, followed by Fisher’s Protected Least Significant Difference test. Differences were considered significant at P < 0.05.

RESULTS

There were no differences in the final body weight among the four groups (Table 2). Groups E + PE, E-PE and W-PE did not differ in food consumption, but intake by the W + PE group was slightly greater (P = 0.0371). Plasma TC was greater in hamsters that did not receive PE than in those that did (Table 3). Plasma TG did not differ among groups but

TABLE 2

Effects of force-feeding red wine phenolic extract (PE) in 2.6 mol/L ethanol (E + PE), red wine phenolic extract in water (W + PE), 2.6 mol/L ethanol (E-PE) or water (W-PE) on body weight and food intake of hamsters fed an atherogenic diet

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>ANOVA P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E + PE</td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>77.4 ± 1.8</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>109.1 ± 2.2</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>4.34 ± 0.100b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8. Data were analyzed by two-way ANOVA followed by the Least Significant Difference test. A difference of P < 0.05 was considered significant. NS, not significant. P ≥ 0.05.

2 Liquid matrix is water or 2.6 mol/L ethanol.

3 Not determined.

TABLE 3

Effects of force-feeding red wine phenolic extract (PE) in 2.6 mol/L ethanol (E + PE), red wine phenolic extract in water (W + PE), 2.6 mol/L ethanol (E-PE) or water (W-PE) on plasma lipid and apolipoprotein concentrations in hamsters fed an atherogenic diet

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>ANOVA P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E + PE</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>7.48 ± 0.30</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>3.59 ± 0.17</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.69 ± 0.08</td>
</tr>
<tr>
<td>Apo-A1, g/L</td>
<td>1.743 ± 0.045</td>
</tr>
<tr>
<td>Apo-B, g/L</td>
<td>0.185 ± 0.006bc</td>
</tr>
<tr>
<td>Apo-A1/Apo-B</td>
<td>9.44 ± 0.38b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8. Data were analyzed by two-way ANOVA followed by the Least Significant Difference test. A difference of P < 0.05 was considered significant. NS, not significant, P ≥ 0.05.

2 Liquid matrix is water or 2.6 mol/L ethanol.
tended \((P = 0.0652)\) to be greater in groups E-PE and W-PE than in groups E + PE and W + PE.

PE was without effect on plasma HDL-C (Table 3), whereas ethanol induced a significant 9.5% decrease \((P = 0.046)\). In contrast, no significant effects of PE or liquid matrix were found for the plasma Apo-A1 concentration. Ethanol and PE interacted to affect plasma Apo-B concentration; PE induced a greater decrease in hamsters fed water \((-40\%)\) than in those fed ethanol \((-7.5\%). PE had a more potent effect when dissolved in water \(+80\%) than in ethanol \(+12\%) in increasing the Apo-A1/Apo-B ratio; ethanol was more efficient as the liquid matrix \(+46\%) than water \(+11\%).

Plasma antioxidant capacity (PAC) is given in Table 4; PE significantly increased the PAC \((P < 0.0001)\) by 9% in Group E + PE and 18% in Group W + PE vs. their respective controls; PAC was also significantly increased by alcohol \((P < 0.0001)\) in hamsters fed PE \(+14\%) and in those not fed PE \(+24\%). No effect of PE was found on liver glutathione peroxidase activity when solubilized in alcohol, whereas it increased enzyme activity 67% when in water \((P = 0.0043)\). Ethanol and PE interacted to affect liver glutathione peroxidase activity \((P = 0.0031)\). Average aortic fatty streak accumulation (AFSA), measured as the percentage of Oil Red O staining relative to the total area surveyed (Table 4), was significantly decreased \((P = 0.0004)\) in hamsters that received PE in ethanol \(-32\%) and in water \(-29\%\); ethanol reduced AFSA \((P < 0.0001)\) in groups that received PE \(-62\%) and in those that did not \(-60\%).

### DISCUSSION

The French paradox (10) was hypothesized to be due to the antioxidant actions of red wine phenolics and their ability to inhibit LDL oxidation in vitro (40). This hypothesis assumed that oxidized LDL play a causative role in atherosclerosis, and more generally, that deleterious oxidation of various sorts contributes to disease development (41).

The aim of the present study was to evaluate the potential of phenolic compounds of red wine, administered in the presence or absence of ethanol, to prevent the progressive alteration of health and the occurrence of early atherosclerotic lesions in hamsters fed a high fat/low antioxidant diet. The effect of ethanol alone was tested for comparison. Associated changes in some plasma lipids and lipoproteins were also explored.

The increase in plasma antioxidant capacity following force-feeding of PE in ethanol (Group E + PE) (Table 4) was in agreement with recent data in humans after ingestion of moderate amounts of red wine (42,43); this increase could be attributed to the wine phenolics absorbed. Compared with the group receiving PE in water \((W + PE)\), this more marked increase of PAC could be explained in part on the basis of the ethanol aiding phenolic absorption (7,42).

PE administered in the form of either reconstituted or alcohol-free wine had beneficial effects, i.e., hypercholesterolemic and to a lesser extent hypotriglyceridemic activities in hamsters because it has been reported that increased cardiovascular risk reflected an increase in circulating cholesterol (44) and triglycerides (45). This cannot be attributed to differences in food or energy intake. We have shown here that this effect was due to the phenolic compounds of wine, in agreement with some of our previous studies using rats fed high cholesterol and a grape seed extract \((46,47)\) and also with other reports relative to tea polyphenols in experimental cholesterol (48) in hamsters fed a high fat diet \((34\) and in humans (49). Plausible mechanisms include the inhibition of cholesterol and bile acid absorption \((46\) and a high output of fecal fatty acids \((34\). Our results clearly demonstrated that consumption of PE in either ethanol or water reduced plasma Apo-B concentration with no effect on Apo-A1 (Table 3). The catabolism of LDL depends on the recognition of cellular receptors by Apo-B; its measurement, and also that of Apo-A1, allows us to assess the risk of cardiovascular disease \((50)\). In considering the development of early atherosclerosis \(<10\%\) foam cell coverage of the aorta), the improvement of the Apo-A1 to Apo-B ratio by PE, ethanol and a combination of both is relevant to a reduced aortic fatty streak area (AFSA) (Table 4).

Hayek et al. (51) observed an attenuation of the development of atherosclerotic lesions in the aortic arch in Apo-E-deficient mice fed red wine or quercetin, or to a lesser extent, in mice fed catechins. It is difficult to compare these results with the present study. However, the mechanisms underlying the antiatherogenic effect of phenolic compounds are not yet clearly understood, although some possible explanations can be found in the literature. Reactive oxygen species intervene in atherogenesis through the induction of endothelial leukocyte adhesion molecule-1 expression \((18)\) and monocyte chemotactic protein-1 \((52)\) in the endothelium. Moreover, a cytotoxic peroxynitrite generated by the reaction between superoxide and nitric oxide reduces the activity of glutathione peroxidase \((53)\) which may play a role in antioxidative status.

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#### TABLE 4

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>E + PE</th>
<th>W + PE</th>
<th>E-PE</th>
<th>W-PE</th>
<th>PE</th>
<th>Liquid matrix</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAC, mmol/L</strong></td>
<td>1.135 ± 0.013</td>
<td>0.993 ± 0.025</td>
<td>1.040 ± 0.016</td>
<td>0.840 ± 0.017</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td><strong>AFSA, % of aortic surface</strong></td>
<td>1.470 ± 0.200</td>
<td>3.890 ± 0.310</td>
<td>2.170 ± 0.230</td>
<td>5.470 ± 0.150</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td><strong>GSHPx, μmol/(mg protein · min)</strong></td>
<td>0.188 ± 0.009</td>
<td>0.248 ± 0.015</td>
<td>0.190 ± 0.014</td>
<td>0.148 ± 0.017</td>
<td>0.0043</td>
<td>NS</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 8. Data were analyzed by two-way ANOVA followed by the Least Significant Difference test. A difference of P < 0.05 was considered significant. NS, not significant, P = 0.05.

2 Liquid matrix is water or 2.6 mol/L ethanol.
This enzyme reduces hydrogen peroxide and organic peroxides to water and organic alcohols, respectively. It has been determined that Se was at the active site of the enzyme (54). As a result of decreased Se availability in a selenium-deficient diet, GSHPx protein levels and activity are decreased (55). Such a situation was mimicked here in Group W-PE, which had a low level of GSHPx activity. Catechins and their related compounds possess potent radical scavenging activity (56) and red wine is highly effective in directly scavenging peroxyl radicals (57); this could be related to the higher enzyme activity in groups receiving ethanol, PE in ethanol or PE in water by a sparing effect. However, it has been reported (58) that chronic ethanol consumption alters GSHPx activity in rat liver after consumption of a diet containing 36% of total energy as ethanol for 31 d. This is not comparable to the moderate quantity of ethanol given to hamsters, but it may explain in part the difference in GSHPx activity between Groups E + PE and W + PE. This may be one mechanism by which PE reduces the development of atherosclerosis in hamsters (i.e., increased plasma antioxidant capacity and liver glutathione peroxidase activity) (Table 4). From this point of view, our data suggest that ethanol is a complementary factor in the benefits of regenerated red wine for hamsters.

Although all of the fluids in this study produced a significant inhibition of atherosclerosis (except water), PE was however more effective in reducing AFSA when given in 2.6 mol/L ethanol rather than in water (−62%) and alcohol was as efficient (−60%) as PE; this could be attributed to a synergistic effect of phenolic compounds and alcohol. In fact, although the effect of 2.6 mol/L ethanol on plasma Apo-B level was unexpected, some authors have attributed a low risk of cardiovascular disease to light alcohol consumption (59,60), and this observation has suggested a protective effect of light drinking; the beneficial effects of moderate quantities of alcohol have been ascribed in part to an inhibition of platelet aggregation (61) through a reduction in the thrombotic tendency (10), contributing to the beneficial effects of moderate alcohol consumption against coronary artery disease (62). Low intakes of alcohol have also been shown to stimulate the production of prostacyclin, a potent vasodilator and platelet antiaggregant (63). Some studies reported a significant beneficial effect of ethanol on atherosclerosis in mice (64,65). More recently, it has been shown (66) that ethanol itself acts as an antioxidant toward in vitro LDL peroxidation initiated by RO2/O2: using a low ethanol concentration added to LDL solutions (4.2 mmol/L), all OH were scavenged by ethanol to give RO2 which are much less efficient initiators of lipid peroxidation than OH; these authors concluded that ethanol only contributes to the beneficial effect of moderate wine drinking with regard to cardiovascular disease, and is separate from the specific antioxidant activity of polyphenols. In addition, the presence of alcohol in wine may improve the availability of phenolics by increasing intestinal absorption, and either by delaying excretion or perhaps by altering its course through xenobiotic excretion pathways (7). Other authors postulated that ethanol prevents the precipitation of polyphenolic tannins in the digestive tract (67). Although alcohol is not a treatment for atherosclerosis, these reports on its effects in antioxidative processes are in agreement with and support our findings.

In our work, we demonstrated that PE efficiently reduces atherosclerosis and that the development of the aortic disease depends on the fluid in which PE was given. More recently, Vinson et al. (68) reported that red wine and dealkoholized red wine decreased early atherosclerosis in hamsters significantly more than ethanol alone. However some experimental conditions were different, i.e., hamsters received a 0.2% cholesterol/10% coconut oil diet, they had free access to solutions for 10 wk and 1.46 mol/L ethanol was used. Moreover, this atherogenic diet was not devoid of antioxidant vitamins and selenium. Despite these differences in experimental conditions, our results concerning the preventive effect of PE are similar, suggesting that phenolics that can induce a direct antioxidant effect may also act by indirect mechanisms (sele- nium enzymes and vitamins E and C economy, metal chela- tion). Indeed, several vitamins and minerals may be involved in metabolic events that protect the vascular endothelium or maintain endothelial integrity. Of particular interest are vitamin E, vitamin C and selenium. Vitamin E is the only significant lipidal-soluble chain-breaking type of antioxidant present in blood and cellular membranes (69) and may play an important role in preventing atherosclerotic lesion formation. Part of its protective mechanism is its ability to protect LDL from lipid peroxidation (70) and thus decrease the recruitment of monocytes into the arterial subendothelium by smooth muscle cells (71). Abundant data from epidemiologic studies suggest that greater intakes of antioxidant vitamins such as vitamin E and vitamin C are associated with reduced risk of atherosclerotic vascular disease (72). Vitamin C is an essential cofactor and an important biological reducing agent. The mechanism by which vitamin C could be protective against atherosclerosis can be attributed to its ability to prevent LDL oxidation (73) and reduce the oxidized vitamin E. Because hamsters synthesize vitamin C, its addition to the diet was deemed unnecessary. In other species, vitamin E and selenium have a synergistic effect on lipid peroxidation mechanisms. Selenium functions as an antioxidant because it is a key component of GSHPx and GSHPx-dependent mechanisms protect cellular components from oxidative stress and damage (74). The presence of phenolic compounds in the diet could extend the beneficial effects of these antioxidants.

Finally, considering their preventive effects against atherogenesis, in moderate quantities, phenolic compounds from PE in alcohol are more efficient than in water or than ethanol alone.

**LITERATURE CITED**


wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis 156: 67–72.